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DEVELOPMENT OF THE PROCARP AND CYSTOCARP IN THE GENUS PTILOTA.

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(WITH PLATES XVIII AND XIX)

THE following account of the structure and development of the procarps and cystocarps in the genus Ptilota is the result of the writer's studies begun in 1892 upon two Pacific coast forms. Later the points then worked out were verified in an Atlantic coast species, *P. serrata*, and this form, for reasons of convenience, was chosen as the type for the detailed description of the anatomy and development of the cystocarpic fruit. The account of *P. plumosa* and *P. plumosa filicina* is of the nature of a comparison with *P. serrata*. Most of the work was carried on in the cryptogamic laboratory of Harvard University under the direction of Dr. Farlow.

PTILOTA SERRATA Kütz.

This species is very abundant on the Atlantic coast north of Cape Cod, and fruiting specimens, either cystocarpic or tetrasporic, may be obtained readily in the proper season. The writer's material has all come from Nahant, Mass., and the cystocarpic plants have been found there as early as March and as late as May. Although cystocarpic fronds are abundant

and covered with fruit in all stages of development, the writer has never succeeded in finding any antheridial plants, although he has carefully searched all sorts of specimens, nor have any such ever been found on this coast to his knowledge.

For convenience the subject-matter of this description will be grouped under the following heads: (*a*) structure and mode of growth of the frond; (*b*) morphology and development of the procarpic branches; (*c*) development of the group of procarps; (*d*) minute structure of the procarps; (*e*) development of the cystocarp. The subjects included in the first two divisions will be treated in the briefest possible manner. At the end of the descriptions will be found a section which treats of the relation of the type of cystocarp found in Ptilota to that of allied genera, and also some remarks upon the physiological character of this method of carposporic reproduction.

STRUCTURE AND MODE OF GROWTH OF THE FROND.

The writer can add nothing to the thorough description of the structure of the frond of this genus first presented by Nägeli (47), and later more exhaustively considered by Cramer (63). However, a short account seems necessary to make clear the morphology of the parts of the fruit.

The branches of the frond, styled pinnæ, consist of a central axial siphon of large oblong cells or segments covered by a thick cortex of small cells. A large apical cell (*fig.* 1, *x*) terminates the axial siphon of the pinna, and by its repeated transverse division new segments are added to the axial siphon.

On the pinnæ, attached to alternate segments of the axial siphon, one on each side, are borne pairs of lanceolate structures called pinnules. In this species the pinnules are usually unequal in size, one being quite small, and their distribution is such that when a certain segment bears its large pinnule on the right hand side of the pinna, the next large pinnule above or below is attached to the left side of a segment. As the small pinnules are borne opposite the large pinnules, upon the same segments, their arrangement is of course exactly the same as the latter.

The segments of the axial siphon which bear the pinnules are called nodes and the segments between them internodes.

The larger pinnule of the pair begins to develop from the node almost immediately after the latter has been cut off from the apical cell. The nodal segment first grows out to one side, the side that is opposite to that of the young pinnule on the node just below it, and a cell is cut off. This cell is the earliest stage of a pinnule. It assumes the rôle of an apical cell, and by successive transverse divisions gives rise to a row of cells which becomes the axial siphon of the pinnule. When the larger pinnule is well under way in its development, the node gives rise to another cell on the opposite side of the pinna, and from this cell is developed the second pinnule, which rarely becomes as large as the first and sometimes remains quite abortive. Stages illustrating the above description may be seen in *fig.* 1.

Cells are cut off laterally from both sides of the axial siphon of the pinnule, and these by successive transverse divisions develop a system of lateral branches. The young pinnule then has the structure of a membranous tissue the thickness of a single cell, but it really consists of an aggregation of filaments, all in the same plane, each of which grows in precisely the same manner as the axial siphons of pinnæ and pinnules, viz., from apical cells.

The three systems of filaments, (1) the axial siphons of the pinnæ, (2) the axial siphons of the pinnules, and (3) the lateral branches from the axial siphon of the pinnules, are the framework which determines the shape of the frond. All other cells are part of the cortex proper. There is no cortex on the young pinnules and at the tips of the pinnæ, but on older portions of the frond its gradual development may be easily traced. Short branches grow up over the axial siphons in older parts, covering them with several layers of cells. This collection of cells constitutes the cortex.

The entire frond then consists of an elaborate system of filaments, and the growth of all parts is strictly from the terminal cells (apical cells) of the branches. A segment cut off from

the apical cell of a filament never divides except to give rise by lateral outgrowths to a new branch of the filament.

MORPHOLOGY AND DEVELOPMENT OF THE PROCARPIC BRANCHES.

The procarps of the genus Ptilota are found in certain specialized portions of the frond called procarpic branches. In *P. serrata* the procarpic branches for the most part take the place of the smaller pinnules usually found on sterile plants. They are therefore attached to the nodes of the pinnæ and situated opposite well developed pinnules. Procarpic branches are sometimes to be found on the edge of the pinnules, but they are not common in this species. The structure of the procarpic branches clearly shows their homology with the smaller pinnules, and their development is so similar that it is impossible to distinguish the younger stages from one another. Each adult procarpic branch contains an axial siphon which consists of from nine to twelve (typically ten) segments. Pairs of short lateral filaments arise from the segments in the same manner as in ordinary pinnules, and as the branch grows older a rather insignificant system of corticating filaments is developed. Several early stages of procarpic branches are shown in *fig.* 1, the cells being shaded. Those attached to the II, III, and IV nodes consist of but a single cell. Later stages are shown attached to nodes V, VI, and VII. A typical adult procarpic branch is illustrated by *fig.* 3, the specimen from which the figure was drawn being situated on the twelfth node of a pinna.

When procarpic branches are found on the pinnules they are seen to take the place of the teeth that usually occur along the edge of these structures, and to be continuations of the lateral branches from the axial siphon of the pinnules.

The extremity of the adult procarpic branch has the same structure whether the latter is situated on a pinna or pinnule. There is always a terminal cell, the former apical cell (*fig.* 3, cell 11), and it is from this that the group of procarps is derived. The apical cell and all structures derived from it are

numbered eleven in the figures because it is usually the eleventh cell of the axial siphon of the procarpic branch.

DEVELOPMENT OF THE GROUP OF PROCARPS.

The group of procarps is always situated at the tip of a procarpic branch. The number is somewhat variable, but typically five. In order that the reader may follow more readily the account of the development of this structure it will be best to describe first the appearance of mature specimens. It is suggested that he glance at *fig. 10* where an adult group of procarps is shown.

There appear in this figure five trichogynes (numbered 11, 10′, 10′ª, 10″ and 10‴), each of which terminates a short branch consisting of three or four cells. Three of these branches are figured; the other two could not appear in this view. Each branch with its trichogyne is a procarp. Three of the procarps, those numbered 11, 10″, and 10‴, are solitary. The other two procarps are peculiar in that they form a pair united together at the basal cells. The three lower cells of the solitary procarps and one of the basal cells of the pair are all united to the terminal segment of the procarpic branch (*fig. 10*, no. 10). The procarps may then be said to form a group around this terminal segment.

The union between the basal cells of the procarps and the terminal segment of the procarpic branch is effected by the strands of protoplasm so generally found between the cells of Florideæ.

Bearing in mind the structure of the tip of an adult procarpic branch we may now consider the development of the group of procarps. A transverse division of the terminal cell of the procarpic branch (*fig. 2*, cell 10) initiates the development of the group of procarps. The division is somewhat oblique, so that the new terminal cell (*fig. 3*, cell 11) is pushed over towards the axis of the pinna, and the curvature of the procarpic branch is thus made more pronounced. This division is really simply a continuation of the apical growth of the procarpic branch, but

we are justified in laying emphasis upon it for the new terminal cell (*fig. 3*, cell 11) now developed immediately into a procarp. Therefore the segment 10 (*fig. 2*) may be said to terminate the procarpic branch, although really its axis continues through this procarp and only ends with its trichogyne. This procarp is the inner one of the group of procarps, the one nearest the pinna, and in all figures it has been numbered 11.

The development of this individual procarp may be taken as the type for all. The cell that gives rise to it (*fig. 3*, cell 11) divides transversely. The upper cell then elongates and also divides, and the terminal division becomes specialized into the trichogyne. The different stages in the development of this procarp are illustrated as follows. A two-celled stage is shown in *fig. 6*, no. 11, and the same condition appears again in *figs. 7* and *8*, except that in both cases the upper cell is much elongated preparatory to the development of the trichogyne. A half grown trichogyne appears in *fig. 9* (numbered 11) and the mature procarp is shown in *fig. 10*. It must be apparent that the position of this procarp, whether to the right or left of the center of the group, depends upon the side from which the procarpic branch is viewed. Its situation is always on the inside of the group, that is the side nearest the axis of the pinna. It is evident that the procarp follows the same type of growth as other parts of the frond. That is, the growth is from the terminal cell, and the structure is a short branch of three cells, one of which becomes specialized into the trichogyne. The number of cells in the different procarps varies, but the method of development is the same in all.

We may now consider the peculiar pair of procarps on the outside of the group. Beginning with the stage shown in *fig. 3*, we see that a cell (no. 10′) has been cut off from the terminal segment of the procarpic branch. This cell quickly develops into a short branch, usually of four cells, which curves inwards somewhat as is shown in the later stage (*fig. 4*). The cells of this branch are short and thick and lie closely pressed against one another. The terminal cell eventually becomes a

trichogyne. However, before this takes place the basal cell of the branch, that which is attached directly to the terminal segment of the procarpic branch (*fig. 4, c*) gives rise to a cell laterally. This condition is shown in *fig. 5*, cell 10′ᵃ. From this last is developed another branch of three or four cells. The two-celled stage is shown in *fig. 7*, and the three-celled condition in *fig. 8*, in both cases numbered 10′ᵃ. By the elongation of the terminal cell of this second branch the structure becomes a procarp. We have now two procarps of three or four cells each, lying side by side, united to the cell that was first cut off from the tenth segment. By examining the later stages of this pair of procarps (*figs. 7, 8,* and *9*) it will be seen that the trichogyne of the first procarp (procarp 10′) develops before the second. In fact it is usually the first of all the trichogynes in the group of procarps to mature.

It is well to call attention now to the fact, which will receive more detailed treatment later in the paper, that the cystocarp of *P. serrata*, in all cases that the writer has examined (some 112 in number), has always developed from the cell at the base of the pair of procarps. This carpogenous cell (*c* in *figs. 3–9*) is the first to be derived from the terminal segment of the procarpic branch, and therefore next to the basal cell of procarp no. 11 is the oldest cell of the group.

There remain to be considered the two procarps that lie between the inner procarp and the pair on the outside. As is shown in *fig. 10*, these two procarps are attached laterally to the terminal segment of the procarpic branch in such a manner that when viewed from the side one appears in front and the other behind this cell. Their development is precisely like that of the procarp on the inside of the group. A cell is cut off first on one side of the terminal segment and then on the other side (*figs. 5* and *6*, cell 10″). Each of these two cells then develops into a procarp of three cells in exactly the same manner as the other procarps develop. This is well shown in the figures of later stages (*figs. 7, 8,* and *9*). These two procarps are the last of the group to mature.

Contemporaneous with the development of the group of procarps is the luxuriant growth of the whorl of short filaments from the segment of the procarpic branch just below the terminal segment. Some of these filaments become the large bracts that surround the mature cystocarp. They develop, as do all the structures of the frond, by growth from the terminal cells of filaments, and *figs. 5*, *6*, and *10* illustrate the appearance of the chief stages.

The typical number of procarps in the group is five, but instances of over or under production are not infrequent. In cases where the number is less than usual the second procarp of the pair is most likely to be absent, and occasionally one or both of the lateral procarps may be suppressed. Examples of over production are more frequent, and perhaps the most common instance is that in which a pair of procarps is found in place of the inner procarp of the group. Sometimes an additional procarp may be attached to the basal cell of the pair. None of these irregularities transgress the law of development that we have advanced, for in all cases the procarps are short branches, the terminal cells of which have become specialized into trichogynes.

MINUTE STRUCTURE OF THE PROCARPS.

Now that we understand something of the development and arrangement of the procarps in the group, we are in a position to consider the minute structure of the cells. The material had been well fixed in chromic acid, and proved excellent for the examination.

In the first place, we may refer to the peculiar structure of the cell-wall often found around the young procarps, which is different from anything that the writer has ever seen described. Unlike the cell-walls on other portions of the frond, which are perfectly homogeneous in structure, the wall is here distinctly differentiated into an inner and an outer zone. *Fig. 4* may be taken as an excellent illustration of this peculiarity. This specimen had been stained with Böhmer's hæmatoxylin, and the

inner zone was much more strongly tinted than the outer. Even in fresh material and unstained specimens, the inner zone appears of a denser consistence. A most interesting peculiarity of this cell-wall is a series of radiating strands which arise from the edge of the inner zone and pass through the outer zone to the outer edge of the cell-wall (*figs. 4, 5, 6*, etc.). These radiating strands stain about as deeply as the inner zone, and appear to be of the same substance. This complex cell-wall is very common around the developing procarps, and is sometimes found, but not in such a characteristic form, at the apical cell of the pinnæ. The peculiar swollen appearance of the outer zone suggests the phenomena of gelatination, and to test this point the writer treated specimens with a hot solution of potassic hydrate. The consistence of the outer zone was quite unaffected by this treatment, instead of swelling or dissolving as substances of a gelatinous nature would have done. The writer was quite unable to obtain a cellulose test (using iodine and sulphuric acid), either with this curious cell-wall or with the ordinary cell-walls of this plant. But there are reasons from its general appearance and reaction towards stains for believing it to be at least closely related to cellulose, if it be not that substance.

Adopting the terminology of Bornet and Thuret we may divide the procarps into three portions: (1) the trichogyne, (2) the carpogenous cell, and (3) the portion of the procarp lying between these two, consisting of one or two cells, which we may call the trichophoric apparatus (*l'appareil trichophorique*).

We know certainly of but one carpogenous cell in the group of procarps, and this is the basal cell of the first procarp of the pair on the outside of the group. However, it is probable that the basal cell of each procarp is morphologically a carpogenous cell. At all events the following remarks on the structure of the cell that does give rise to the cystocarp are equally true of the basal cells of all procarps. The carpogenous cell at the time when the trichogyne is mature is the largest one in the procarp. It is slightly tinted with the red color of the Florideæ, but a well defined chromatophore cannot be made out dis-

tinctly. The central portion of the cell is a cavity containing cell sap, and the protoplasm with the irregular chromatophore forms a layer next the cell-wall. There is a distinct nucleus imbedded in the protoplasm, and as a rule a well defined nucleolus is apparent in specimens stained with hæmatoxylin (*figs. 11* and *12, c*). The carpogenous cell is connected below with the terminal segment of the procarpic branch, and above with the cell of the trichophoric apparatus, by a strand of protoplasm at each end.

The trichophoric apparatus consists of one or two cells according as the total number of cells in the procarp is three or four. There is a distinct nucleus in each cell, and the general appearance of the cell contents is very similar to that of the carpogenous cell, *i. e.*, the protoplasm containing more or less of the red pigment lies next the cell-wall and encloses a vacuole. In *figs. 11*, *12* and *13* the cell of the trichophoric apparatus is lettered *ta*. The position of the nuclei in the cells of the procarps has been shown in many of the figures. In some of the specimens (*figs. 5–10*) the stain was eosin, in others (*figs. 11–16*) the stain was hæmatoxylin.

The structure and development of the trichogyne now remain to be considered. This organ is very small and delicate, in this species of Ptilota measuring from 40–70μ long and 4μ wide in the thinner upper portions. The base of the trichogyne ("carpogonium," as Schmitz applied the term) is about as wide as the cell of the trichophoric apparatus directly under it, but it grows narrow very rapidly and runs into the very delicate and attenuated upper portion. The base of the trichogyne is not at all swollen, nor is there any constriction between it and the upper portion. The cell contents are hyaline in living specimens, and quite homogeneous. Stains do not bring out any differentiation of the protoplasm aside from a granular structure in the lower portion, and the writer has never seen anything that could be interpreted as a definite nucleus.

Such peculiar cytological structure of the trichogyne cell merits a farther examination, and the gradual development and withering of the organ will now be described. Starting with

the earliest stage, we find a cell at the end of a procarp closely attached to the cell of the trichophoric apparatus (*fig.* 11, *t*). Such a cell contains no distinct nucleus, but the cell contents often show a certain degree of differentiation into vacuoles and aggregations of granular matter. This cell begins to elongate, and as it does so carries up with it the substance of the inner zone of the cell-wall. Finally it pushes through the outer zone of the cell-wall (*fig.* 12), and then simply elongates until the full size is reached. The cell-wall of the upper portion of the trichogyne is composed entirely of the substance of the inner zone, the outer zone remaining around the base of the trichogyne as a sort of collar (*fig.* 13). As the trichogyne elongates the cells-contents become more homogeneous, until aside from some granular matter in the base of the structure there is no differentiation of the protoplasm. The trichogyne is united to the cell of the trichophoric apparatus by a narrow strand of protoplasm.

The first indication that the trichogyne is about to wither appears in the formation of a cap like layer of cellulose, staining deeply with hæmatoxylin, over the cell of the trichophoric apparatus, severing the protoplasmic connection between these two structures. An early stage in the differentiation of this cap is shown in *fig.* 14, and a later stage in *fig.* 15. Contemporaneously with the formation of this cap begins the disintegration of the trichogyne, and this latter process is always associated with the development of a zooglœa of rod-shaped bacteria (*figs.* 14 and 15), with sometimes Leptothrix and Beggiatoa filaments around the ends of the trichogynes. The end of the trichogyne gradually collapses, and the cellulose wall appears to gelatinize, for the outline becomes vague and at last we cannot distinguish the end in the mass of slime. The contents of the trichogyne either disappear entirely, or there are left only small masses of organic matter in the basal portion of the structure.

While the trichogyne is withering the cell of the trichophoric apparatus usually begins to push out at one side of the base of the trichogyne, and assuming the functions of an apical cell it converts the procarp into a filament of several cells that forms

one of a whorl of small bracts around the cystocarp. These filaments with the remains of the trichogynes at one side are frequently met with, and appear in some of the figures illustrating the development of the cystocarp.

We may say at this point that we have never seen any bodies attached to the trichogynes that could be identified as antherozoids. Such observations must be made before the trichogynes begin to wither, as then the bacteria and slime put a stop to all examination of this point. Sometimes the group of procarps contains much foreign matter around the trichogynes, but much of the writer's material was quite clean, and it seemed impossible that the presence of antherozoids should escape notice, yet such material was covered with developed fruit.

DEVELOPMENT OF CYSTOCARP.

We have already stated that the cell at the base of the pair of procarps is the carpogenous cell (*figs. 4–11, c*). It is very curious that the cystocarp should be developed so uniformly from a particular cell, and yet this proved true of every specimen that the writer examined. This cell is one of the first of the cells composing the group of procarps to be formed, and consequently is one of the oldest at the time when the cystocarp begins its development. It is likewise associated with the procarp that as a rule is the first of the group to mature. The development of the cystocarp was studied almost exclusively from serial sections cut from paraffin, the specimens being stained *in toto* with Mayer's acid hæmalum, and on the slide with eosin.

The earliest stage of the cystocarp is frequently met with It consists of a large cell rich in protoplasm, and containing a prominent nucleus, situated in the midst of the group of procarps and united to the carpogenous cell of the outer pair. A glance at *fig. 16* will make plain what is meant. The large cell numbered 10 is the terminal segment of the procarpic branch On the left side of the figure drawn in detail is one of the procarps of the outside pair, and from its carpogenous cell (*c*) has

arisen the first cell of the cystocarp (x). On the right side of the figure drawn in outline, only the position of the nuclei being indicated by shading, are the remains of some of the other procarps of the group with the basal portions of their withered trichogynes. Whenever dotted lines appear in the figures, they mean that the structures indicated were present in the section of the series next the one from which the drawing was made.

The carpogenous cell does not give rise to this first cell of the cystocarp until the trichogyne has begun to wither, and is therefore entirely separated from the cell of the trichophoric apparatus. The first cell of the cystocarp increases in size until it quite fills up the space between the procarps, and then by a transverse division it cuts off a small cell at its base. (*fig. 18*). The lower cell takes no further part in the development of the cystocarp; the upper cell gives rise to the lobes of the favella.

At this point it may be well to consider the possibility of there being cross-fusion between any of the cells of the procarps and those of the young cystocarp. The cells of the young cystocarp are separated from all the cells of the procarps by walls which stain heavily, as has been indicated in *fig. 17*. In none of the many specimens examined was there any indication of the presence of ooblastema filaments or of fusion processes budded out from any cell of the procarps. As the sections were serial the relation of all the cells of the procarps and cystocarps to one another might be studied, and it seems to the writer quite impossible that there could be any connections formed between any of the cells that would not appear on the slides.

The favella consists of a variable number of lobes, from two to five, which as a rule are in widely different stages of maturity. They are quite separated from one another, but are all attached to the second cell (cell x^2 in *figs. 19* and *20*) of the cystocarp. A lobe develops in the following manner. The second cell of the cystocarp pushes out in the form of a pear shaped process that becomes cut off as a cell. This cell by forward growth and a few irregular divisions gives rise to a short filament of thick

segments (*fig. 19*). Branches arise from these segments in profusion and secondary branches from the first, so that ultimately there results an oval body consisting of roundish cells, closely packed together, and yet really constituting a system of filaments. As the lobe matures the connections between the cells are severed and finally they separate as carpospores, quite distinct from one another. *Fig. 20* shows a section through a maturing cystocarp. Here there are three lobes shown in section and the attachment of two of them to the second cell of the cystocarp (x^2) is evident. The largest lobe was made up of ripe spores which were about ready to escape from the cystocarp; the other lobes were much younger. The remains of the procarp (*ta*) with the base of the trichogyne may be seen on the right of the figure.

As the cystocarp develops it frequently happens that the strands of protoplasm between the terminal cell of the procarpic branch and the carpogenous cell and between this last and the first cell of the cystocarp become much wider than they were originally. There is evidently an absorption of the cell-wall between these cells. In *fig. 20* the cell-wall between the terminal segment of the procarpic branch (no. 10) and the carpogenous cell (*c*) has been so far absorbed that were it not for the fact of a nucleus being present in the carpogenous cell, and its position in reference to the procarp and cystocarp, one would be likely to consider it a part of the terminal segment.

PTILOTA PLUMOSA C. AG. AND P. PLUMOSA FILICINA FARL.

The material upon which this examination is based was collected by the author in the month of July 1892 at Pacific Grove, California. In the following account of the structure and development of the procarps and cystocarps of this species and its variety we take it for granted that the reader is familiar with the main points of the account of *Ptilota serrata.* Accordingly the subject is considered under the same divisions and in the same order as those of the preceding description, and the

remarks will be in the nature of a comparison of these two Pacific coast forms with *P. serrata*.

STRUCTURE AND MODE OF GROWTH OF THE FROND.

The structure of the frond of *P. plumosa* and its variety *filicina* is in all essentials identical with that of *P. serrata*. The differences that exist are purely minor peculiarities of size and shape of pinnules and pinnæ, color, habit, etc. The structure of the framework upon which the corticating filaments are laid is quite the same in both species, and the method of growth of all parts of the frond is absolutely identical in the two forms.

MORPHOLOGY AND DEVELOPMENT OF THE PROCARPIC BRANCHES.

There is a more luxuriant production of fruit in the Californian species than on *P. serrata*. While procarpic branches are not rare on the pinnules of *P. serrata*, they are very commonly so situated in *P. plumosa* and *P. plumosa filicina*, and the greater part of the fruit is to be found on those portions of the frond. The procarpic branches on the pinnules, from one to five in number, are usually situated along the inner edge of that structure, where they take the place of the teeth found along the edge of sterile pinnules. The procarpic branches of the Pacific coast forms are shorter than those of *P. serrata*, in *P. plumosa* consisting of only five or six segments, and in *P. plumosa filicina* of eight or nine segments, the number of course being somewhat variable. The procarpic branches of the variety *filicina* are not only longer, but also stouter than in the typical form *plumosa*, in keeping with the coarser texture of the frond. Occasionally a procarpic branch will itself bear procarpic branches, that is, a lateral branch from the axial siphon, instead of developing into a vegetative filament, will give rise to a short branch upon the end of which a group of procarps will be developed.

DEVELOPMENT OF THE GROUP OF PROCARPS.

The group of procarps in its development follows exactly the same steps in *P. plumosa* and its variety as in *P. serrata*. A com-

parison of *fig. 22* with *fig. 7* will show that the two groups of procarps are identical in all the essentials of structure. There was no tendency towards an increase of the typical number of procarps in the Californian plants, but frequently the full number was not present.

The appearance of the pair of procarps on the outside of the group requires a word of notice. The second procarp of the pair is sometimes very small, and its position such that the question might arise as to whether it really is a filament or a number of cells cut off from the basal cell by radial divisions (*fig. 21*). In several such cases, specimens were treated with lactic acid and ammonia, when by carefully crushing the specimen and manipulating the cover glass, the two procarps were separated at all points excepting where the second joined the first at the basal cell. After such treatment it was apparent that the two procarps were distinct branches.

A very exceptional case was observed in the presence of a single procarp on the frond near the base of a pinnule, and in no way connected with a procarpic branch. It was attached to one of the lateral branches of a pinnule of *P. plumosa*, and consisted of three cells, the trichogyne projecting beyond the edge of the pinnule. This was the only exception noted to the rule that in the genus *Ptilota* the procarps are borne at the ends of procarpic branches.

MINUTE STRUCTURE OF THE PROCARPS.

With the general agreement in structure that we have found to exist between the different portions of the frond of the California plants and *P. serrata*, we should hardly expect to find great differences in the minute structure of the procarps. There are no essential differences in the structure of the carpogenous cells and the trichophoric apparatus. The trichogynes of *P. plumosa* and *P. plumosa filicina* are somewhat longer than in *P. serrata*, measuring about 62 μ long and 3 μ wide above the trichophore. In most cases it was quite impossible, after carefully staining, to make out any differentiation of the protoplasm

in this organ beyond a slight granular structure. However, the writer did find occasionally a body in the narrower portion of the trichogyne that had something of the appearance of a very small nucleus. There was a tendency toward a differentiation of the cell-wall around the procarps, manifest in the manner in which the upper portions of the trichogyne arose from a sort of collar, but the writer observed nothing that could be compared with the complex cell-wall of *P. serrata*.

No antherozoids were found attached to the trichogynes, and as yet no antheridial plants of this Pacific coast species have been found. However, the writer did not make the same determined search for male plants in this species as he did in the case of *P. serrata*.

DEVELOPMENT OF THE CYSTOCARP.

There is a perfect agreement in the structure of the cystocarp of *P. serrata* and the two forms we are considering. Not only do the lobes of the favella arise in the same manner, but they are developed from the same cell in both cases, this cell being the second cell of the cystocarp.

There does not appear to be the same uniformity as to the position of the carpogenous cell in *P. plumosa* and its var. *filicina* as in *P. serrata*. Out of thirty-five specimens of cystocarps examined, twenty-nine were developed from the basal cell of the pair of procarps on the outside of the group, the homologue of the carpogenous cell of *P. serrata*; four cystocarps came from the basal cell of the procarp on the inside of the group, and in two instances they had arisen from the terminal segment of the procarpic branch.

Three figures of different stages of the cystocarps have been introduced, which make clear certain points about their development that are not shown in the illustrations of *P. serrata*. In *fig. 23* we have an instance where the carpogenous cell (*c*) of the procarp on the inside of a group has pushed out towards the center and contains two nuclei. This the writer considers to be the earliest stage in the development of a cystocarp. The dis-

tinct nuclei of the cells of the trichophoric apparatus (*ta*) are shown, and above them the trichogyne, which has just begun to wither, may be seen. In *fig. 24* we have the one-celled condition of a cystocarp, and the specimen is of particular interest because the chromatin of the nucleus is very well defined, having apparently gathered together into the chromosomes preparatory to nuclear division. *Fig. 25* illustrates beautifully the manner in which a new lobe (b^1) arises from the second cell (x^2) of the cystocarp when an older lobe may be well along in its development.

COMPARISON OF THE TYPE OF PROCARP AND CYSTOCARP OF PTILOTA WITH THOSE OF ALLIED GENERA.

We have fortunately very good descriptions of the types of procarps and cystocarps of the genera most closely allied to Ptilota. The following have been carefully studied: Callithamnion,[1] Pterothamnion,[2] Griffithsia,[3] Ceramium,[4] Lejolisia,[5] Spermothamnion,[6] Ptilothamnion,[7] and Spondylothamnion.[8]

There are many differences in the precise cell arrangements of the procarps in the genera just mentioned, each having its peculiarities, and in none of them are the conditions very much like those of Ptilota. However, in the following two cases certain resemblances are worth noting.

In *Callithamnion elegans* Schousb., according to Bornet and Thuret (76), one of the segments of a branch gives rise to a cell from which is developed a three-celled procarp, the basal

[1] *Callithamnion corymbosum* Lyngb. Bornet and Thuret (67) 145; Thuret (78) 67. *pl. 33–35*. *C. tetricum* Ag. Janczewski (77) 117. *C. elegans* Schousb. Bornet and Thuret (76) fasc. 1: 32. *pl. 10*.

[2] *Pterothamnion plumula* Näg. Schmitz (83) 23, 24.

[3] *Griffithsia corallina* Ag. Janczewski (77) 122. *G. Bornetiana* Farl. Smith (96) 35.

[4] *Ceramium decurrens* Harv. Janczewski (77) 120.

[5] *Lejolisia Mediterranea* Born. Bornet and Thuret (67) 148.

[6] *Spermothamnion flabellatum* Born. Bornet and Thuret (76) fasc. 1: 27, *pl. 9*. *S. hemaphroditum* Näg. Janczewski (77) 115.

[7] *Ptilothamnion pluma* Thuret. Bornet and Thuret (76) fasc. 2: 179, *pl. 46*.

[8] *Spondylothamnion multifidum* Näg. Bornet and Thuret (76) fasc. 2: 182, *pl. 47*

cell of which is the carpogenous cell. Often this segment from which the procarp is developed gives rise to one or two cells that are ordinarily vegetative, but that sometimes become changed into procarps. When this is the case a group of procarps results somewhat resembling the group in Ptilota. The cystocarp consists of several lobes, but unlike Ptilota they all arise directly from the carpogenous cell.

In the genus Ceramium, according to Janczewski, there are found two procarps connected with one carpogenous cell. In Ptilota the pair of procarps situated at the outside of the group appears to have but one carpogenous cell. However, the manner in which the procarps of Ceramium develop is quite different from that of Ptilota, and a morphological relationship seems very unlikely.

REMARKS ON THE CHARACTER OF THIS TYPE OF CARPOSPORIC REPRODUCTION.

Physiologically considered there is a great resemblance between the type of carposporic reproduction of Ptilota and of the several genera previously mentioned. They all agree in that the carpogenous cell is separated from the trichogyne by a trichophoric apparatus consisting of one or more cells. This characteristic of the type is very important from a physiological standpoint, and so considered it matters little what is the precise number and arrangement of the cells of the trichophoric apparatus. Furthermore, if the writer is not mistaken in his interpretation of what has been published by the different writers on the subject, in the genera above named and also in the case of the species of Ptilota studied by him, no actual fusion of the base of the trichogyne with the carpogenous cell has been observed. In most of these genera and also in Ptilota the trichogyne is so far removed from the carpogenous cell that fusion would hardly be possible, except through the agency of an ooblastema filament. However, in spite of very careful search on my part no such filament could be found in Ptilota, nor have I seen in the literature any figure showing an ooblastema filament or any

explicit statement on the part of botanists that they have ever observed one in any of the genera just mentioned.

An exception to the above statement may perhaps be found in some remarks in a recent paper by Professor Fr. Schmitz,[9] in which he expresses the belief that the hitherto accepted accounts of the fertilization in Callithamnion are incorrect. It may be gathered from this statement of his opinion that he was inclined to believe that ooblastema filaments or their equivalent exist in Callithamnion, but the brevity of the account there given and the absence of figures prevents my comparing the complicated condition of things there described with what I have observed in Ptilota.

To bring clearly before the reader the conditions that make a satisfactory explanation of the fertilization of the carpogenous cell in Ptilota so difficult let us examine some of the figures. In the specimen shown in *fig. 24* the trichogyne had clearly begun to wither, and the carpogenous cell was in process of division. *Figs. 15*, *17*, and *24* illustrate the one-celled stage of the cystocarp, the trichogyne in all instances having withered to a certain degree. *Figs. 18* and *19* show later stages of the cystocarp with the withered trichogynes at one side of the procarps, and in *fig. 20* we have a section of an adult cystocarp that illustrates very well the relation between the cell of the trichophoric apparatus (*ta*) and the cell of the cystocarp when the latter is mature.

A glance at these figures must make it apparent that the trichogyne is so far distant from the carpogenous cell that fusion with it would hardly be possible except by means of an ooblastema filament. The writer has never seen anything to indicate the presence of such a filament, and it does not seem to him possible that such a structure could be present and escape notice in serial sections such as he had to study. There was

[9] *La Nuova Notarisia*, III.—: 114. 1892. The first view of Professor Schmitz (see *Untersuchungen über die Befruchtung der Florideen* 23, 24) was that in the majority of the Ceramieæ there is direct fusion between the base of the trichogyne and the carpogenous cell, brought about by the bending of the trichophoric apparatus so that the trichogyne is brought into close proximity to the carpogenous cell.

likewise no evidence of fusion between the cells of the developing cystocarps and the cells of any of the trichophoric apparatuses or the trichogynes. As is shown in all the figures, the cells of the cystocarp are separated from the trichophoric apparatuses by walls of considerable thickness, and cross-fusion of any sort certainly ought to have appeared in the sections. The fact that the sections were serial enabled the writer to examine all sides of the specimens, and would seem to have prevented the possibility of an ooblastema filament or fusion process escaping notice because it lay in such a plane that it could not appear in the median section. However, to guard against error of method the writer crushed out many of the young cystocarps in lactic acid, thus separating the procarps from the central developing cystocarp, and in such specimens saw no indication of an ooblastema filament.

A satisfactory explanation of a sexual process in the case of Ptilota must then be one which answers the following question: viz., How can a sexual impulse be transmitted from a trichogyne to a carpogenous cell when the two structures are separated by a trichophoric apparatus of at least one cell (often more) through which the impulse must pass? From the literature it certainly seems as if the conditions above mentioned were essentially the same in the genera Callithamnion, Griffithsia, Ceramium, Spermothamnion, Spondylothamnion, and Lejolisia, but the writer cannot in most cases speak from a personal study of the forms.

Accepting the dictum that biology now lays down as to the requirements of a sexual act, there must be a transmission of nuclear substance from the antherozoid through the trichogyne to the cells of the trichophoric apparatus, and thence on to the carpogenous cell. Any explanation of sexuality which satisfies the above condition must base its argument upon the fact of there being a continuous mass of protoplasmic matter from the trichogyne to the carpogenous cell, because of strands of protoplasm connecting the cells one with another.

The difficulties that a satisfactory hypothesis must overcome, even though it rest on the above mentioned fact of an

unbroken passage from trichogyne to carpogenous cell, are very great. It must postulate a process, the complexity of which, if the writer is not mistaken, is not to be found in the sexual reproduction of any organism. So far as the writer is able to judge, the union of sexual elements in both the animal and plant world is facilitated as much as possible by simplicity of conditions, *i. e.*, the two elements are given every opportunity to unite directly, and the direct union of the protoplasmic masses of two cells is the characteristic phenomenon of a sexual act. In this case it is necessary to assume the transmission of nuclear substance through cells which are themselves nucleated, and apparently are not specialized for this purpose, at least they are not materially different in structure from ordinary vegetative cells. The evidence upon this last point, it will be remembered, was that the cells of the trichophoric apparatus after the withering of the trichogynes increase in size and frequently give rise to a small filament or bract, thus showing that they have not lost the potentialities of vegetative cells. The passage of nuclear substance from one cell to another by way of one or more cells would be a fact quite contrary, the writer believes, to the usual conception of the individuality of the cell. Botanical science as yet furnishes no instance of such a phenomenon.

The writer carefully studied the cells of the trichophoric apparatus, endeavoring to find indications of a change in appearance before and after the development of the cystocarp, but in the many specimens he examined there was nothing to indicate a change of structure of the cells themselves, and nothing was ever seen that could be interpreted as nuclear substance *en route* to the carpogenous cell.

It must be apparent to the reader that we have to deal with a very difficult problem. From the observations here recorded an explanation of sexuality in this genus must overcome some serious obstacles. Investigators in this field of study have always considered that the sexuality of the Florideæ was an established fact. Yet in this genus the cytological conditions of the procarps are such that it is difficult to conceive the mech-

anism by which the nuclear substance of the antherozoid could be carried to the female cell. But to make the problem still more complex there is the fact that the antherozoids are apparently rare, if not wanting, and yet cystocarpic fruit is very abundant. From the present examination, somewhat unsatisfactory as including only two species, the writer cannot but think it very probable that the cystocarp in this genus develops non-sexually.

The evidence in favor of a theory of apogamy may be briefly summarized as follows:

1. The entire absence of bodies attached to the trichogynes that could be identified as antherozoids impressed the writer as being very significant.

2. Cystocarpic plants of *P. serrata* and *P. plumosa* with its variety *filicina* are common and bear immense quantities of fruit, there being as a rule a cystocarp at the end of every abortive pinnule (procarpic branch), and sometimes borne along the edge of the pinnules. Immense quantities of antherozoids, particularly as they are non-motile in the Florideæ, would be required to insure the development of such a profusion of cystocarps arranged in such a regular manner upon the frond, yet no antheridial plants of *P. serrata* or *P. plumosa* have been reported. It is natural to expect that antheridial plants will be found, as has been the case with *Ptilota elegans* Bonnem, but they ought to exist in great quantity to produce such a profusion of fruit if the cystocarp is to develop as the result of a sexual act.

3. The uniformity with which the cystocarp is developed from one carpogenous cell in the case of *P. serrata* and one of two cells in the case of *P. plumosa* can be explained in two ways. Either the cell has been specialized as the female cell, of which there is no evidence in its structure or position, or it is the cell which by virtue of its age and situation is best fitted to give rise to the fruit apogamously. As has been pointed out, the carpogenous cell is one of the oldest in the group of procarps, and perhaps for that reason it may be the cell strongest in potentialities, best prepared to develop the fruit. At all

events the uniformity of the position of the carpogenous cell adds another difficulty to be explained by a theory of sexuality, while it is but reasonable to suppose that when a plant adopts a method of apogamous development of its fruit certain cells, because of position or age affording perhaps greater nourishment, would be best fitted to undertake reproductive functions.

4. The absence of facts pointing to a fertilization of the carpogenous cell through the trichophoric apparatus, and the difficulty of understanding such a process, while affording simply negative evidence on the subject, nevertheless deserves attention, and appears to the writer as a point in favor of the hypothesis of apogamy.

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EXPLANATION OF PLATES XVIII AND XIX.

All figures sketched with the Abbé camera: *fig. 1*, × 300; *figs. 2* and *3*, × 800; *figs. 4–15*, × 1100; *figs. 16–22*, × 800; *figs. 23–25*, × 1100.

Ptilota serrata Kütz.

FIG. 1. End of a pinna; *x*, apical cell; I–VII, nodes; early stages of procarpic branches shaded.

FIG. 2. An adult procarpic branch from the twelfth node; stained with eosin.

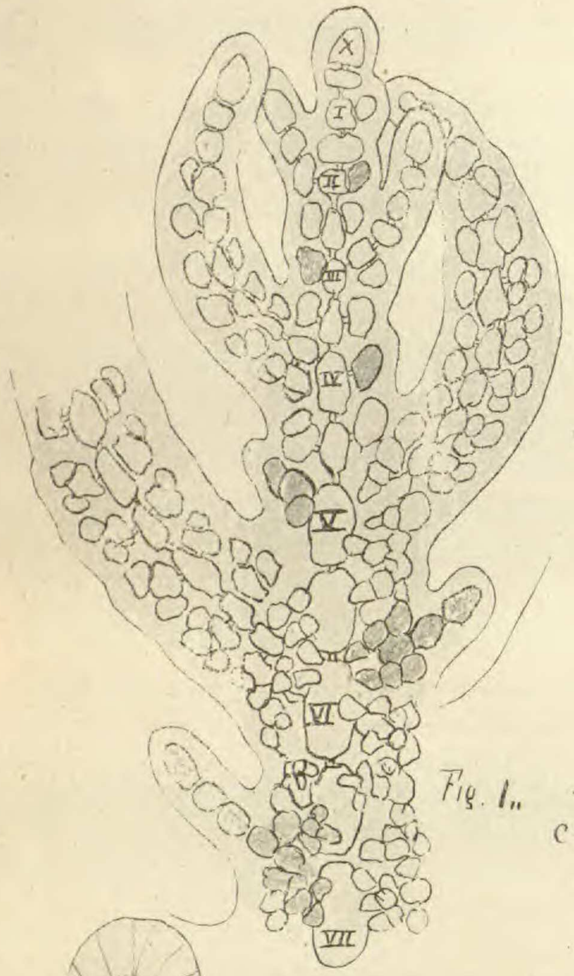


Fig. 1.

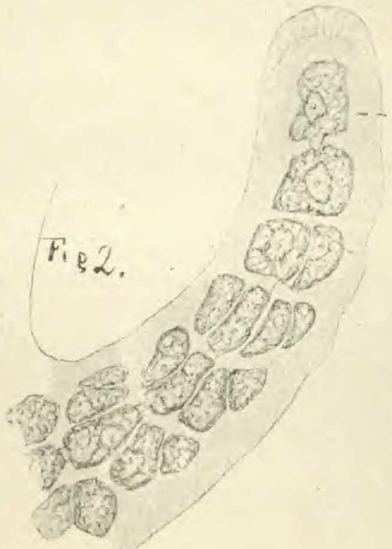


Fig 2.

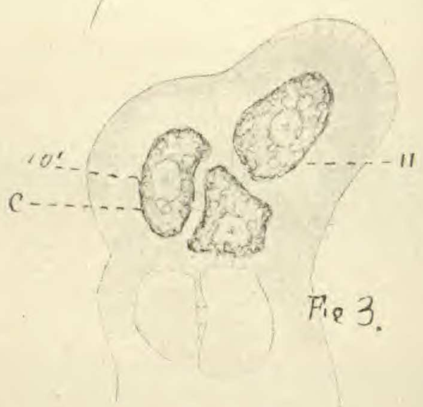


Fig 3.



Fig 4



Fig 5

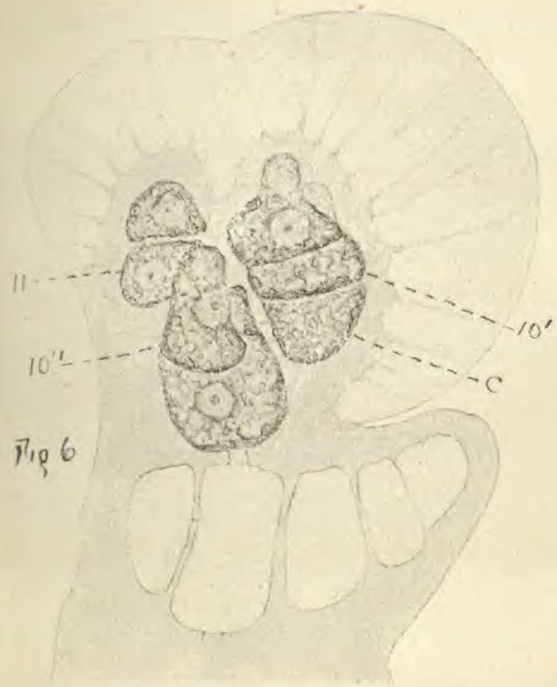


Fig 6

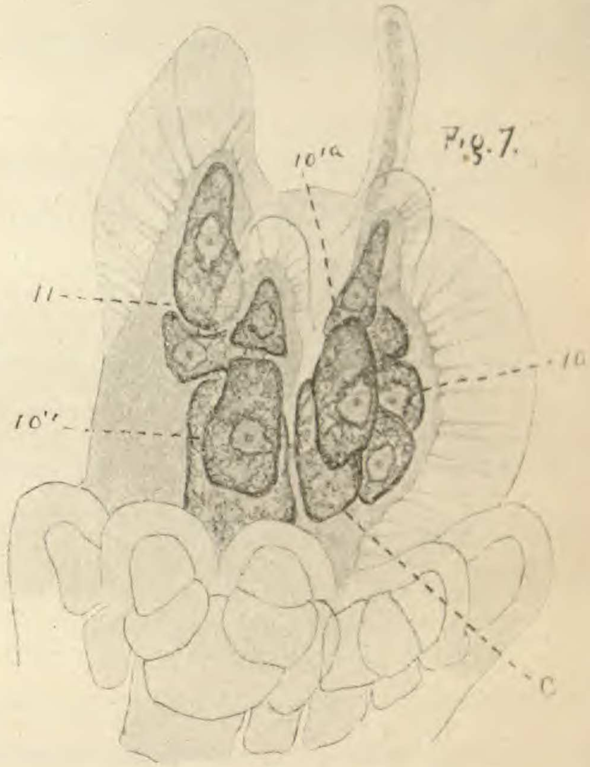


Fig. 7.

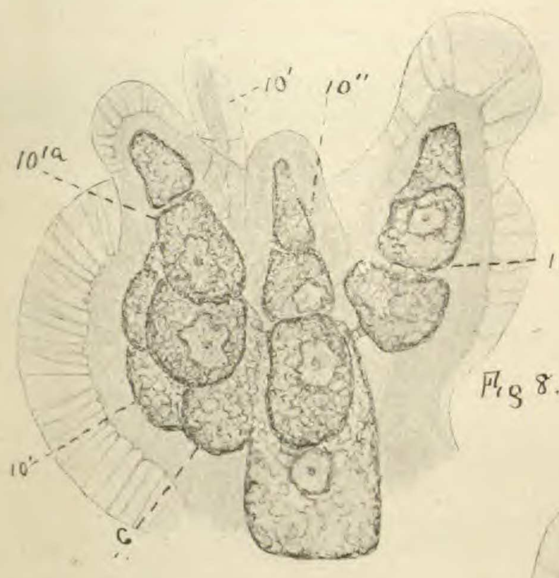


Fig 8.

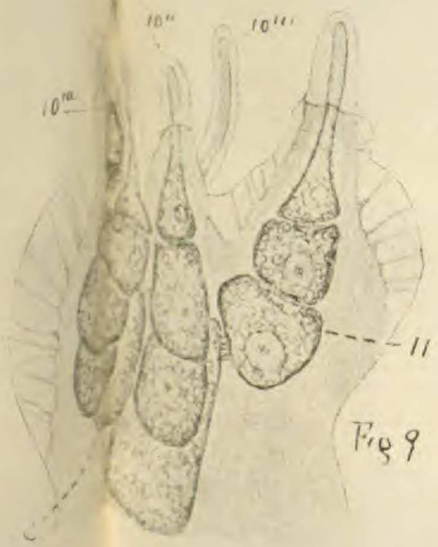


Fig 9

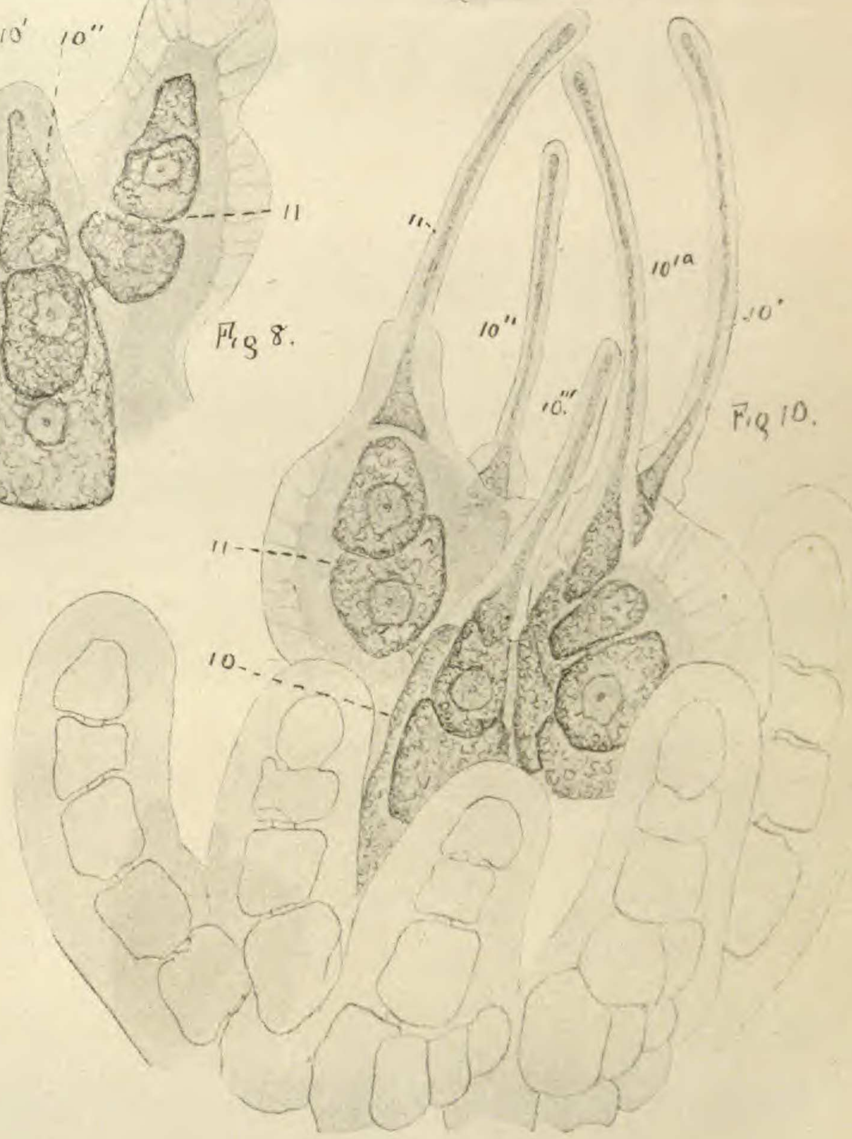


Fig 10.

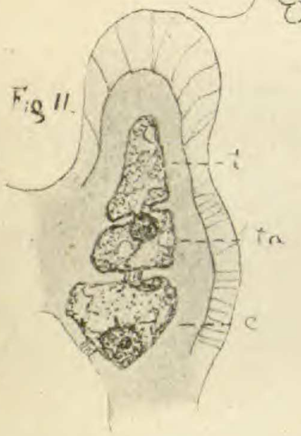


Fig 11.

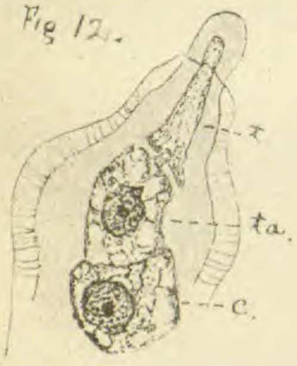


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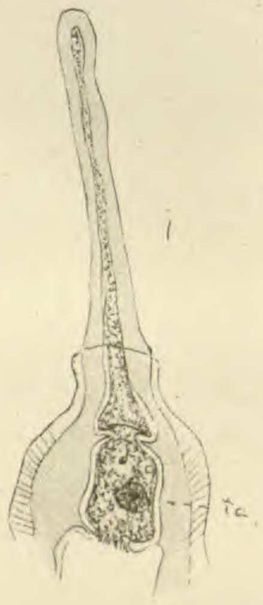


Fig 13.



Fig 14.

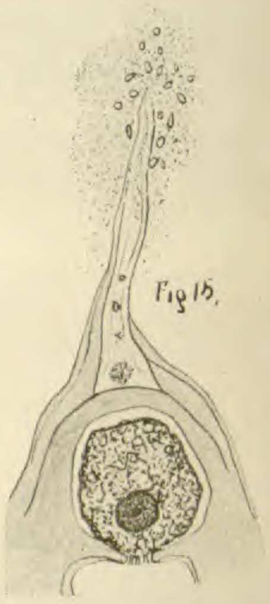


Fig 15.

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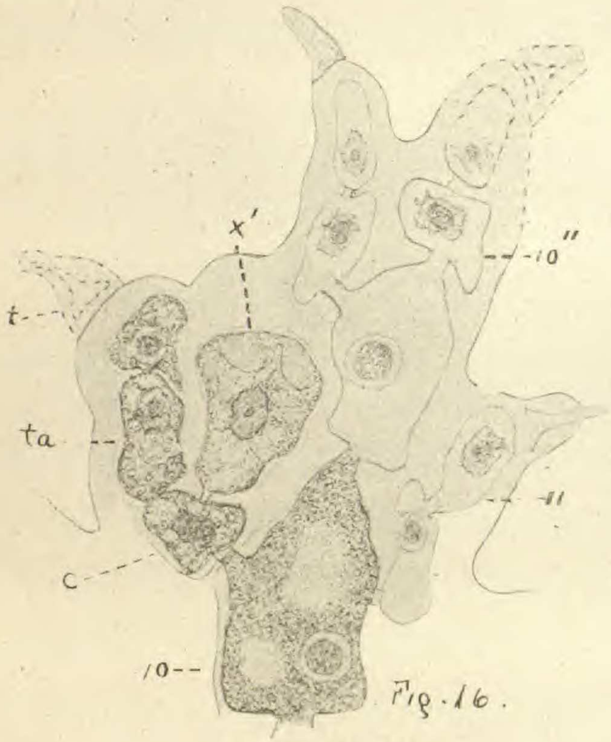


Fig. 16.

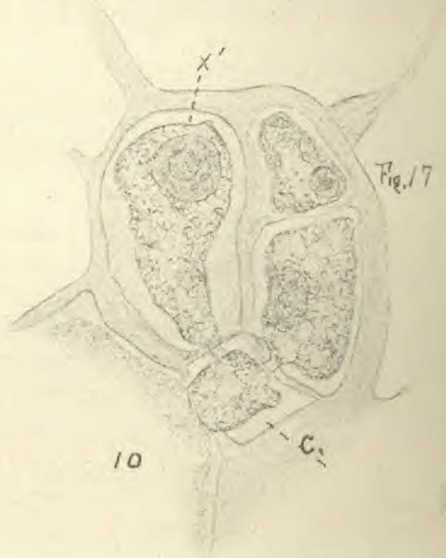


Fig. 17.

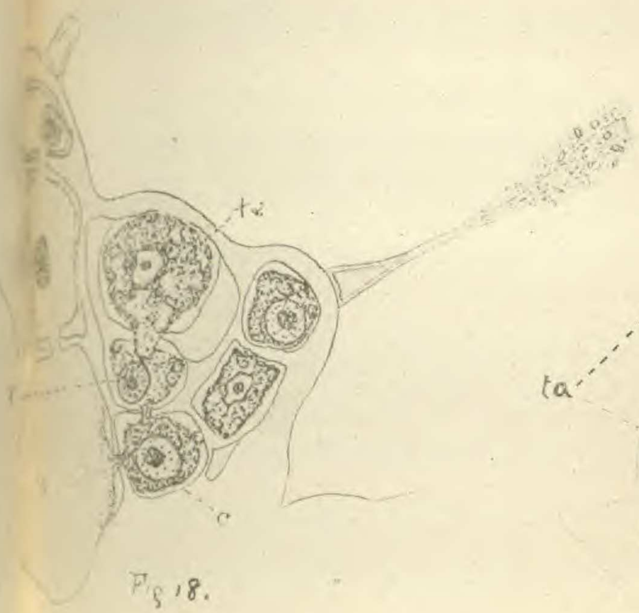


Fig. 18.

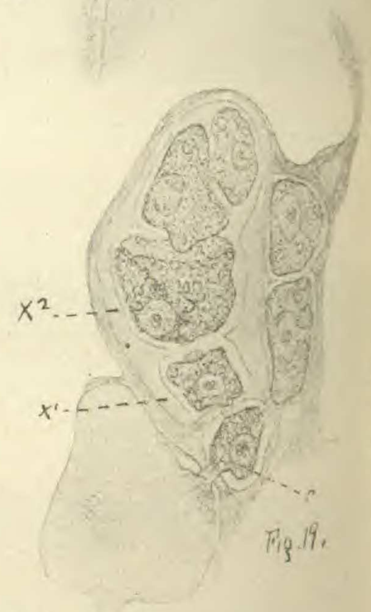


Fig. 19.

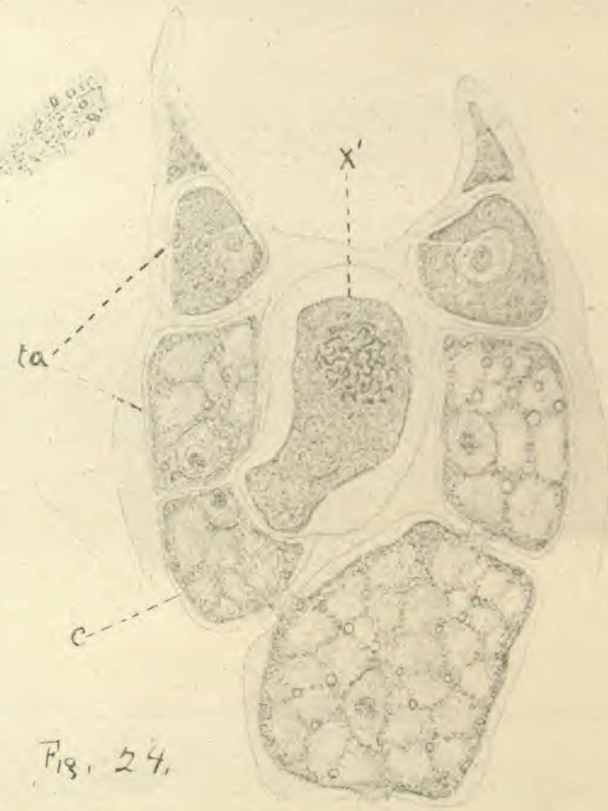


Fig. 24.



Fig. 22.

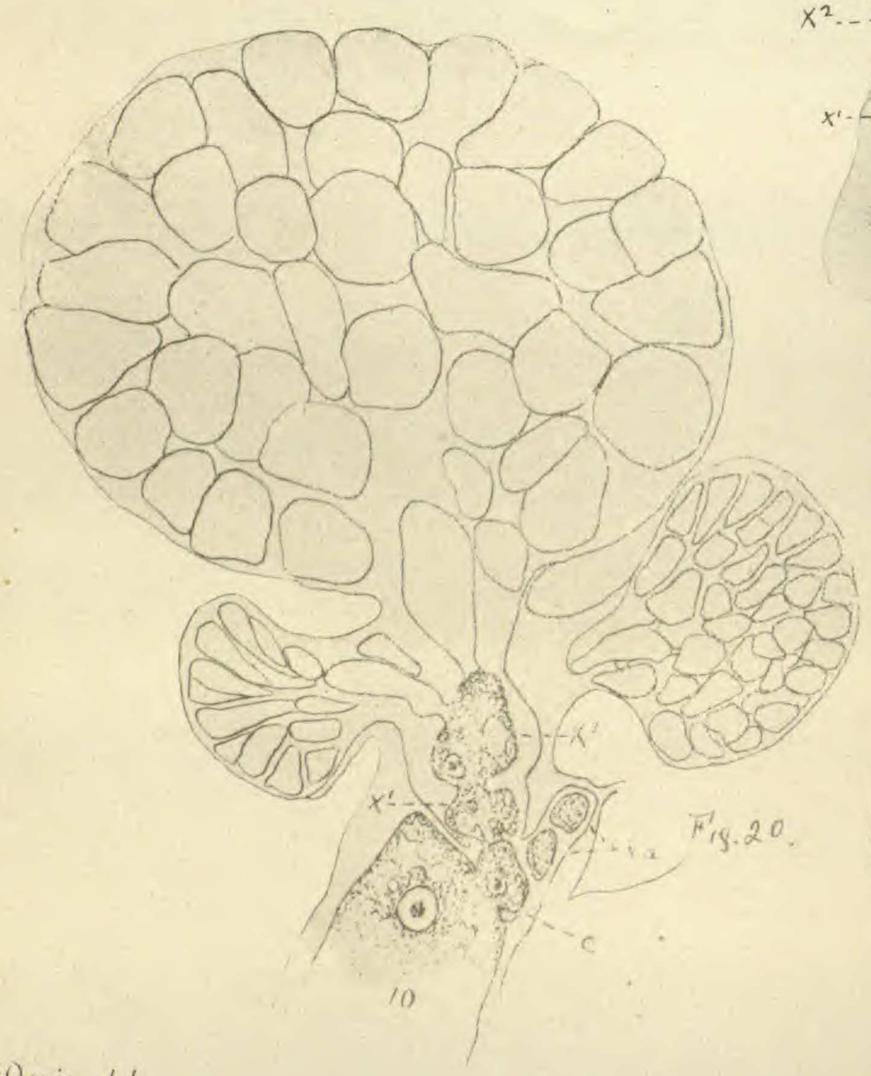


Fig. 20.

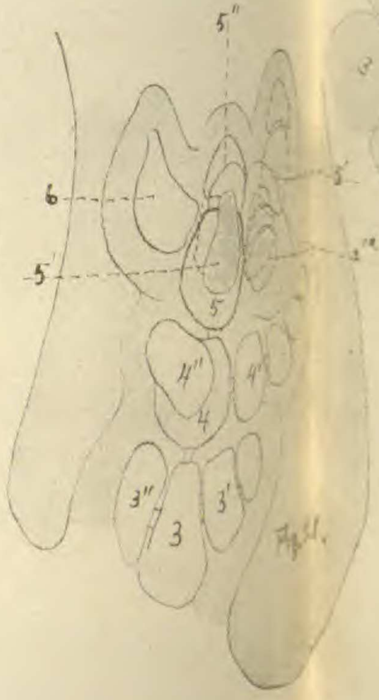


Fig. 21.

Fig. 23.

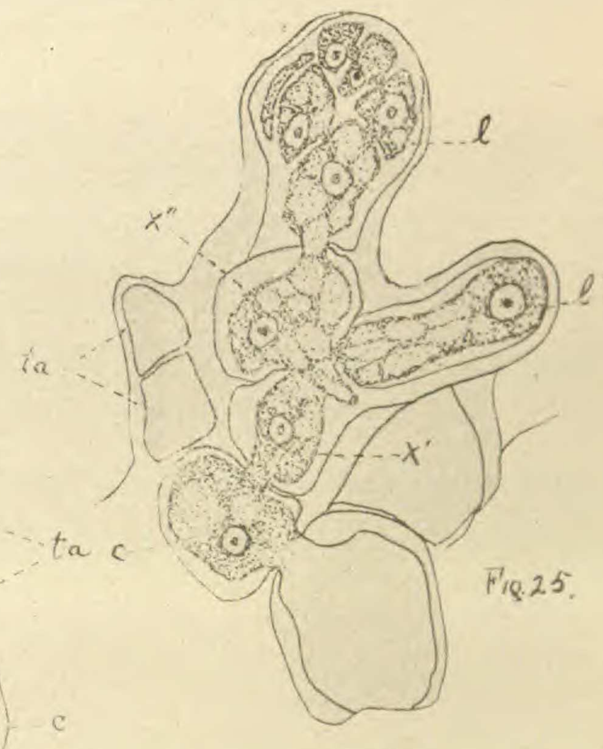


Fig. 25.

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DAVIS & PTILOTA